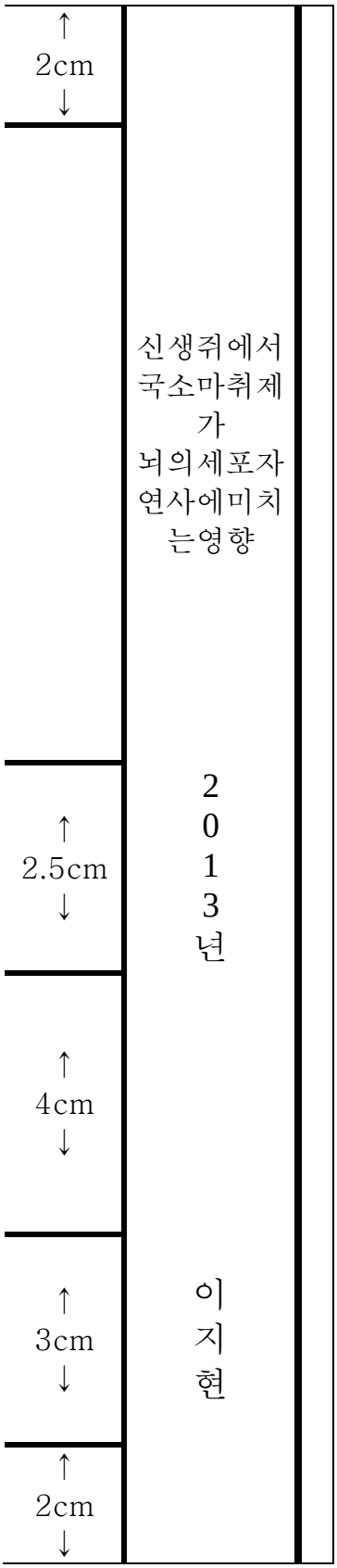
의학석사 학위논문

The Effect of Local Anaesthetics on Apoptotic Neurodegeneration in the Developing Brain in Mice

신생쥐에서 국소마취제가 뇌의 세포자연사에 미치는 영향

2013 년 2 월

서울대학교 대학원
의학과 마취통증의학과 과정
이 지 현


↑
2cm
↓
신생쥐에서
국소마취제
가
뇌의세포자
연사에미치
는영향
↑
2.5cm
↓
2
0
1
3
년
↑
4cm
↓
↑
3cm
↓
이
지
현
↑
2cm
↓

↑
2cm
↓

The Effect of Local Anaesthetics on Apoptotic Neurodegeneration In the Developing Brain in Mice

↑
2.5cm
↓

2013

↑
4cm
↓

Lee
Ji Hyun

↑
3cm
↓

↑
2cm
↓

The Effect of Local Anaesthetics on Apoptotic Neurodegeneration in the Developing Brain in Mice

지도 교수 김 진 태

이 논문을 의학석사 학위논문으로 제출함
2012년 10월

서울대학교 대학원
의학과 마취통증의학전공
이 지 현

이지현의 의학석사 학위논문을 인준함
2012년 12월

위 원 장 _____ (인)
부위원장 _____ (인)
위 원 _____ (인)

The Effect of Local Anaesthetics on Apoptotic Neurodegeneration in the Developing Brain in Mice

by
Lee Ji Hyun

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Master of Science in Meidicine (Anesthesiology and Pain Medicine) at Seoul National University College of Medicine

October 2012

Approved by Thesis Committee:

Professor _____Chairman

Professor _____Vice chairman

Professor _____

ABSTRACT

Introduction: General anaesthetics induce the neuronal apoptosis in the immature brain. Regional anaesthesia using local anaesthetics (LA) can decrease the dose of general anaesthetics or even be an alternative to general anaesthesia. However, no report has examined the effect of LA on brain apoptosis. Therefore, this study investigated the effect of LA on neuronal apoptosis.

Methods: Fifty-one 7-day-old C57BL6 mice were allocated into control (group C), lidocaine (group L), lidocaine plus midazolam (group LM), and isoflurane (group I) groups. Group C received normal saline administration. Groups L and LM were injected with lidocaine (4 mg/kg, subcutaneously) and with midazolam (9 mg/kg, subcutaneously). Group I was exposed to 0.75 vol% isoflurane for 6 h. After 6 h, apoptotic neurodegeneration was assessed using caspase-3 immunostaining and TUNEL staining.

Results: For the entire brain, neuronal cells exhibiting caspase-3 activation were observed more frequently in group LM and group I than group C (P = 0.039 and 0.002). In the thalamus, apoptosis of group L was significantly more than that of group C (P = 0.001), but less than group I (P = 0.002). In the cortex, the mice of group I experienced more apoptosis than the mice of group L and C (all $P < 0.001$). In the caudate nucleus, apoptosis of group LM

was more observed than that of group L (P = 0.006) and C (P = 0.002). On TUNEL staining, the number of cells per mm^2 undergoing DNA fragmentation in group C was less than in groups LM and I (P = 0.006 and P = 0.002, respectively). Apoptosis of group I was also more observed than that of group L (P = 0.036). More apoptosis occurred in group L than in group C, but the difference was not significant (P = 0.05).

Conclusions: LA may induce neuronal apoptosis in the immature brain, although not to the same degree as inhalational anaesthetics. Adding midazolam to lidocaine has an effect on neuronal apoptosis similar to that of inhalational anaesthetics. More research regarding the degree and exact mechanism of brain apoptosis caused by LA is needed.

Keywords : local anaesthetics, brain, apoptosis

Student number : 2011-21853

CONTENTS

List of Figures

Introduction

Recent animal studies have demonstrated that early exposure to general anaesthetics or sedatives induces apoptotic neurodegeneration in the developing brain and results in memory/learning impairment [1-5]. In addition, several clinical studies have suggested that general anaesthesia in childhood is associated with poor neurodevelopmental outcomes [6, 7]. Therefore, the need for less neurotoxic alternatives is increased in children.

Local anaesthetics (LA) act mainly by inhibiting sodium influx through sodium-specific ion channels. Since the mechanism of LA differs from that of general anaesthetics, LA may have different effect on neurons in the developing brain. If regional anaesthesia using LA can reduce neuronal depletion compared with general anaesthesia, it would be an alternative method to avoid general anaesthetic-induced neurotoxicity in some surgery, such as on the hand, foot, or superficial lesions. To our knowledge, no study has examined the effect of LA on apoptotic neurodegeneration in the developing brain.

In children, it is difficult to perform surgery under regional anaesthesia only. A sedative such as midazolam is usually combined with regional anaesthesia. Therefore, the effect of the combination of LA and a sedative on neurotoxicity should also be evaluated.

The purpose of this study was to evaluate the effect of LA on neuronal apoptosis, with or without midazolam, and to compared this effect with that of

inhalation anaesthetics in the developing mouse brain.

Materials and methods

Animals and Anaesthesia Treatment

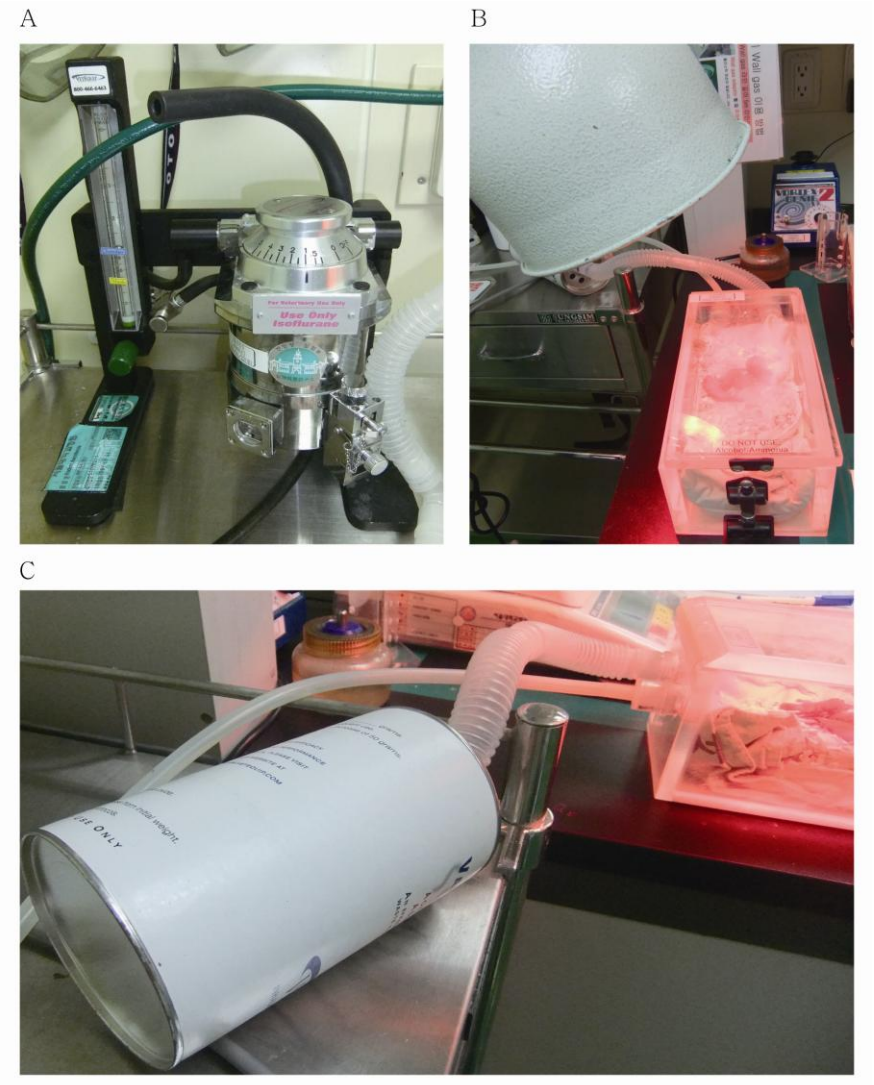
The experiments were approved by the International Animal Care and Use Committee of Seoul National University (No. 11-0232). Fifty-one 7-day-old (p7) C57BL6 mice weighing 2.5–3 g were studied because the peak of neurosynaptogenesis occurs at this age, and they are most sensitive to neuroapoptosis caused by anaesthetic agents such as GABA-mimetics or NMDA antagonists [8]. The mice were randomly divided into the control (group C, n=15), lidocaine (group L, n=12), lidocaine plus midazolam (group LM, n=14), and isoflurane (group I, n=10) groups.

Group C was given normal saline (10 μL/g) subcutaneously (sc), as a protocol of previous experiment [9], using a 30-G needle. In group L, lidocaine was injected at dose of 4 mg/kg sc, which is a typical dose used for regional nerve blocks in humans. Mice in Group LM received lidocaine 4 mg/kg sc and midazolam 9 mg/kg sc. The dose of subcutaneous midazolam was based on previous studies [4, 8]. After administration of normal saline sc as in group C, mice in group I were placed in the anaesthesia chamber. Isoflurane 0.75 vol% was delivered to the chamber with 100% oxygen for 6 h (Fig. 1). The drugs were diluted to adjust the volume so that it equalled that of the normal saline given to group C. A heat lamp was applied in order to avoid hypothermia throughout the experiment. External stimulation was given to the mice in the group I if the breathing rate was decreased 20% below the

baseline.

After single injection of neurotoxic agents, neuroapoptosis response of brain reached peak at 5-6 h post-treatment in previous studies [8, 10]. Therefore, six hours after drug administration, all pups were decapitated and the brains were extracted within 20 seconds and fixed with 10% paraformaldehyde (pH 7.4) for 48 h.

Figure 1. Experimental setting of isoflurane anaesthesia



(A) Isoflurane vaporizer for rodents. (B) Mice of group I were in transparent chamber and warmed by a heat lamp for the maintenance of body temperature. (C) The chamber has two ports, one for connection to vaporizer and the other for connection to carbon dioxide absorbents.

Immunohistochemical (IHC) study of activated caspase-3 (C-3A)

Vibratome sections were washed in deionised water, quenched for 5 min in a solution of 3% H_2O_2 for endogenous blocking, and incubated for 30 min in rabbit anti-active caspase-3 antiserum (D175, Cell Signaling Technology, Beverly, MA, USA) diluted 1:500 in blocking agent. Following incubation with D175 primary antibody, the sections were buffered and incubated for 30 min in secondary antibody (Dako EnVision+ System- HRP-labelled Polymer, anti-rabbit, Dako, Denmark). Immunostaining was visualised using DAB chromogen (Dako, Denmark) and counterstaining with Mayer's haematoxylin. Positive staining was recognised under a light microscope as a diffuse brown colour in the cytoplasm.

TUNEL staining

To detect DNA fragmentation, TUNEL staining was performed with the ApopTag® Peroxidase *in situ* Apoptosis Detection Kit S7100 (Millipore, Billerica, USA). After deparaffinising with xylene and graded concentrations of alcohol, the brain sections were exposed to Proteinase K for 15 min at room temperature. Endogenous peroxidase activity was quenched with 3% H_2O_2 in phosphate-buffered saline (PBS) for 5 min at room temperature. Then, the sections were incubated with terminal deoxynucleotidyl transferase (TdT) in a humidified chamber at 37℃ for 1 h. After incubation with anti-digoxigenin conjugate for 30 min at room temperature, Peroxidase Substrate (0.05% diaminobenzidine, DAB) was applied to develop colour. Then, the

specimens were washed with distilled water and counterstained with 0.5% methyl green for 10 min at room temperature. TUNEL-positive cells were deemed to be undergoing apoptosis.

Cell counting

The sagittal sections of brain were used for evaluation of apoptosis [8, 10, 11]. The brain was cut 100 μm from the midline in each hemisphere and sectioned sagittally in 4-μm slices. For quantitative counts of C-3A positive cells, the first sagittal section from each hemisphere was selected. PC-based image analysis software (LAS AF, Leica Microsystems CMS GmbH, Germany) interfaced with a Leica DM2500 microscope via a Leica DFC280 digital camera (Leica Microsystems Imaging Solutions Ltd. UK) was used for image analysis.

As previously described [8], a C-3A-positive cell was defined as a neuron with dendrites or those larger than 8 μm. The total number of C-3A-positive cells in the entire area of two sagittal sections was counted and divided by the total area of the two sections determined using the LAS software to calculate profile densities. In addition, the densities were calculated for specific regions, such as the thalamus, cortex and caudate nucleus.

Two additional sagittal sections, which were also symmetric, were selected for TUNEL staining. The number and density of TUNEL-positive cells in the entire viewing field were obtained.

Quantitative analysis including cell counting was performed using Leica QWin imaging software (Leica Microsystems, Germany), which can

distinguish apoptotic cells by recognising different RGB patterns, and the results were checked by an expert who was blinded to the treatment.

Statistics

All data were analysed using SPSS statistics ver. 19 (SPSS, Chicago, IL, USA). Before analysis, normality test were performed. If data were normally distributed, the density of apoptotic cells were analyzed using one-way ANOVA and Dunnett method for post hoc analysis. If data did not follow normal distribution, Kruskal–Wallis test was performed and Mann-Whitney *U* test with Bonferroni corrections was used. A $p < 0.05$ was considered to indicate statistical significance.

Results

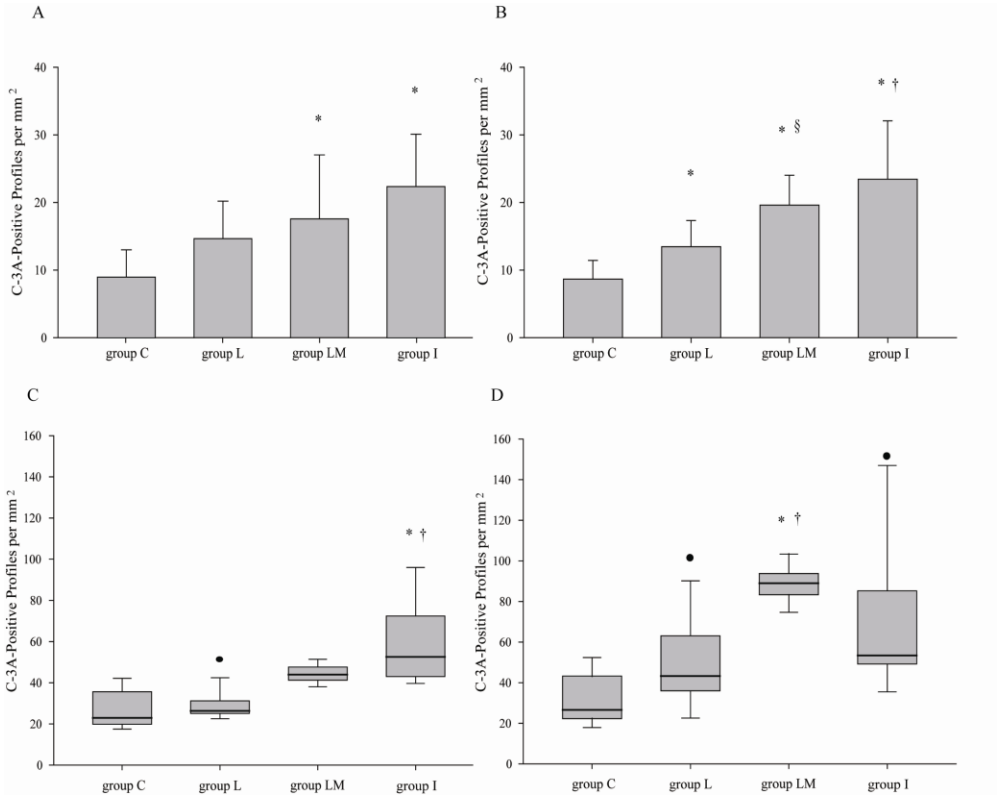
Total 51 C57BL6 p7 mice were included in this study. The data from entire brain and the thalamus were normally distributed. Whereas the data from the cortex and caudate did not follow normal distribution.

Imunohistochemical (IHC) study of activated caspase-3 (C-3A)

By IHC for C-3A, all mice underwent neuroapoptosis to varying degrees and apoptosis was identified in all brain regions, especially the thalamus, cortex and caudate nucleus. Figure 2 shows the densities of apoptotic cells in the entire brain and each individual region. For entire brain, neuronal cells exhibiting caspase-3 activation were observed more frequently in group LM and group I than group C (P = 0.039 and 0.002). Otherwise no significant difference between groups was observed.

There were some differences of the results between in the entire brain and the specific regions. In the thalamus, apoptosis of group L was significantly more than that of group C (P = 0.001), but less than group I (P = 0.002). In the cortex, the mice of group I experienced more apoptosis than the mice of group L and C (all $P < 0.001$). In the caudate nucleus, apoptosis of group LM was more observed than that of group L (P = 0.006) and C (P = 0.002). Figures 3 to 5 show the different densities of cells undergoing apoptosis in the cortex, caudate nucleus, and thalamus, respectively.

Figure 2. The density of apoptotic cells in the entire brain and each individual region in IHC study of activated caspase-3



A: the entire brain, B: the thalamus, C: the cortex, D: the caudate nucleus

In A and B, the data were normally distributed and one-way ANOVA was used for comparison of apoptosis in each group. The data of C and D did not follow normal distributions, and Kruskal–Wallis test was performed and Mann-Whitney *U* test with Bonferroni corrections was used.

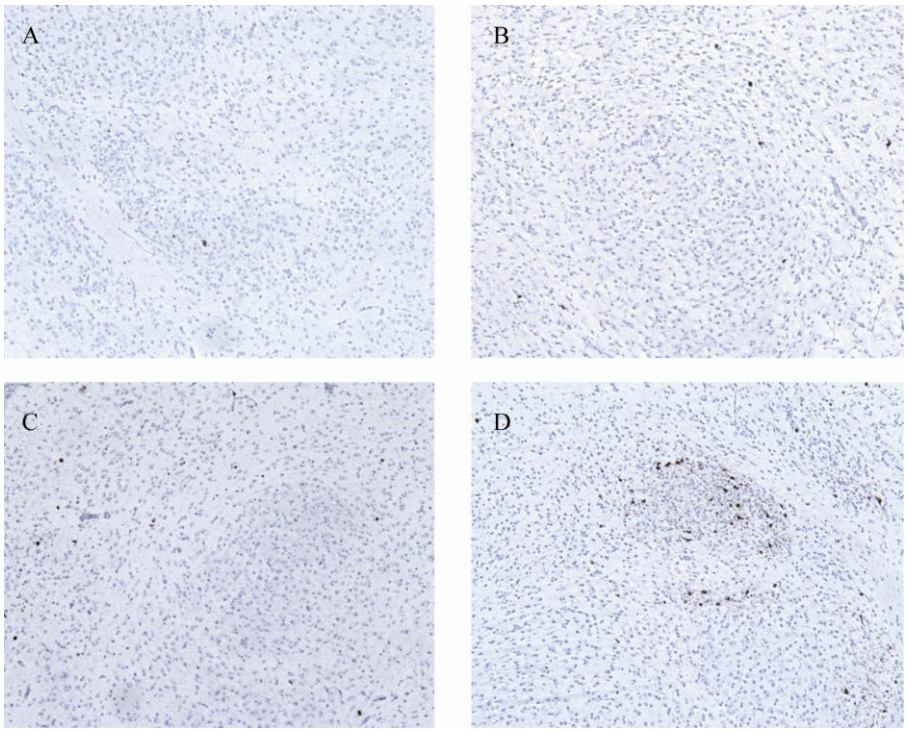
IHC: immunohistochemical

*$P < 0.05$ compared with group C and † $P < 0.05$ compared with group L in A and B

§ $P = 0.05$ compared with group L

*$P < 0.0083$ compared with group C and † $P < 0.0083$ compared with group L in C and D

Figure 3. Apoptosis in the thalamus confirmed by caspase-3 immunostaining

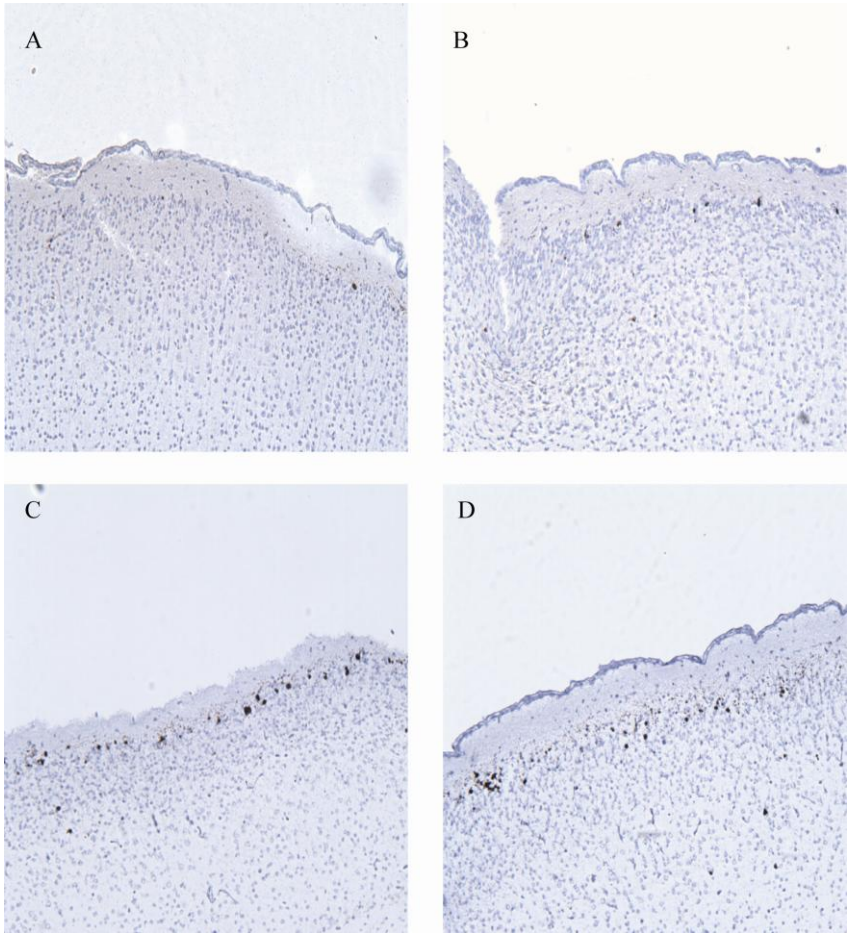


Dark brown-colored cell bodies are in process of apoptosis. There were more neuronal apoptosis in group I and LM when compared with group C. Also, group I exhibited more apoptosis than group L. C-3A positive cells of group L is significantly more observed than that of group C.

A: group C, B: group L, C: group LM, D: group I

C-3A: activated caspase-3

Figure 4. Apoptosis in the cortex confirmed by caspase-3 immunostaining

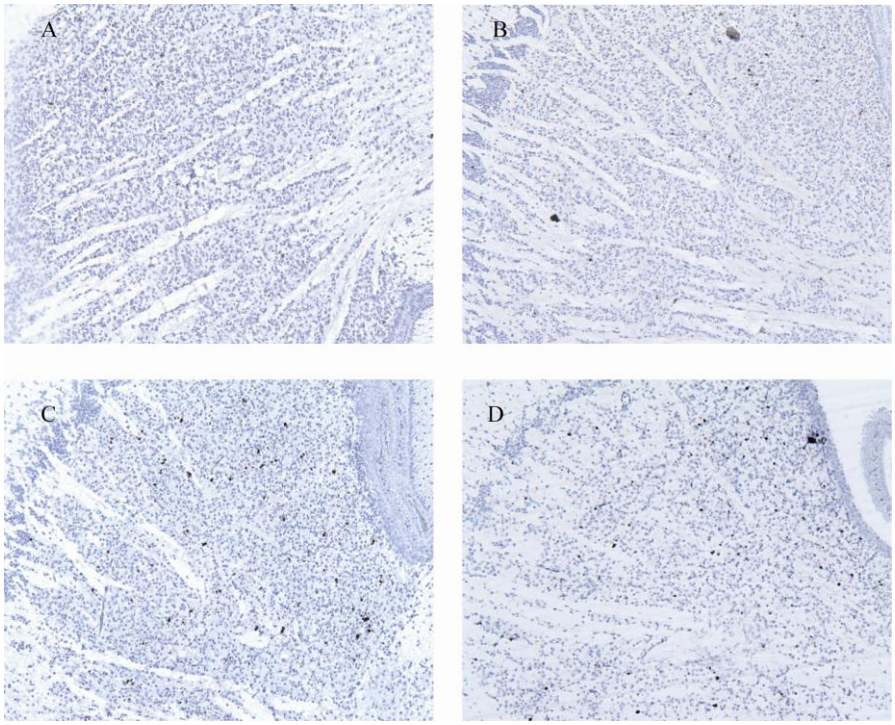


Dark brown-colored cell bodies are in the process of apoptosis. Statistically, C-3A positive cells of group I is significantly more than that of group C and group L.

A: group C, B: group L. C: group LM, D: group I

C-3A : activated caspase-3

Figure 5. Apoptosis in the caudate nucleus confirmed by caspase-3 immunostaining



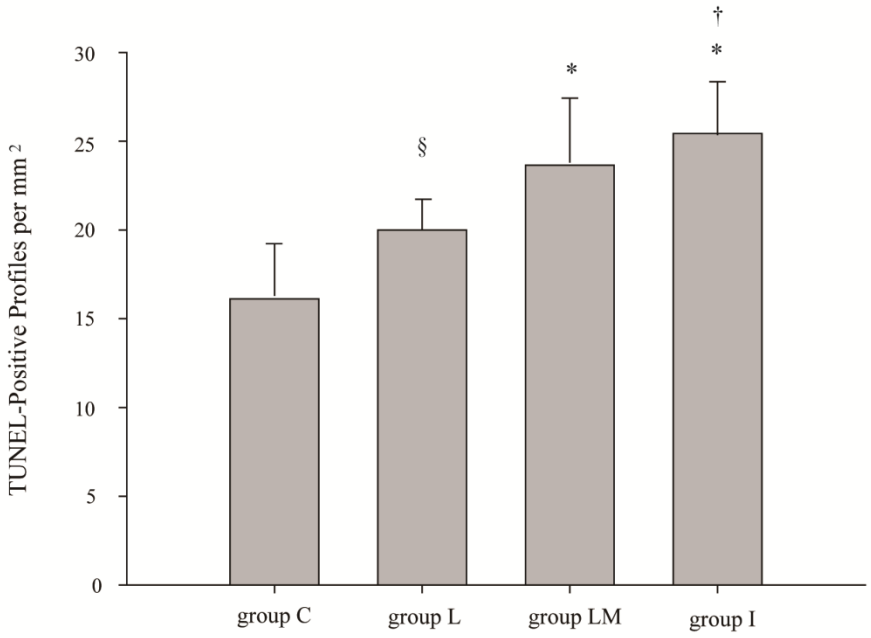
Dark brown-colored cell bodies are in process of apoptosis. Apoptosis of group LM was more observed than that of group L and group C

A: group C, B: group L. C: group LM, D: group I

TUNEL staining

On TUNEL staining, the number of cells per mm^2 undergoing DNA fragmentation in group C was less than in groups LM and I ($P = 0.006$ and $P = 0.002$, respectively). Apoptosis of group I was also more observed than that of group L ($P = 0.036$). More apoptosis seemed to occur in group L than in group C, but the difference was not significant ($P = 0.05$). There was also no significant difference in the number of apoptotic cells between groups L and LM, and group LM and I (Figure 6).

Figure 6. Total density of apoptotic cells of each group in TUNEL staining



The number of cells per mm^2 undergoing DNA fragmentation in group LM and group I were significantly more than that of group C. Apoptosis in group I was also more occurred than group L.

*P < 0.05 compared with group C

† P<0.05 compared with group L

§ P = 0.05 compared with group C

Discussion

General anaesthetics that act through GABA and NMDA receptors cause apoptotic neurodegeneration in the brain [1-5]. However, no study has investigated the association between LA and brain apoptosis. Therefore, we evaluated the effect of LA on apoptotic neurodegeneration in the vulnerable immature brain.

The anaesthetic doses used to cause apoptosis were selected based on previous experiments. The dose range of 6-h-isoflurane inhalation was from 0.75–1.5 vol% in p7 mice and rats [3, 12]; however, all four mice in the 1.0 vol% isoflurane chamber died in our pilot study. Therefore, we chose an isoflurane concentration of 0.75 vol% during the experiment. Nikizad *et al*.[4] reported that isoflurane 0.75 vol% was clinically relevant. Midazolam 9 mg/kg sc induced significant neuroapoptosis in infant mice and was considered a sedating or sub-anaesthetic dose for infant mice and equivalent to the dose for a human infant [4, 8]. For lidocaine, it was important to find an adequate dose that did not exceed the toxic dosage. A previous experiment used subcutaneous lidocaine 6-10 mg/kg for evaluation of wound healing, and that dosage did not induce any significant adverse event in adult mice [13]. In one study of lidocaine and its beneficial effect on focal ischaemia in the rodent brain, lidocaine was administered as an intravenous bolus (1.5 mg/kg) followed by an intravenous infusion (2 mg/kg/h) for 165 min [14]. Since the allowable maximal dose of lidocaine for a regional nerve block is 5 mg/kg in

infants and children [15], we chose a single bolus dose of 4 mg/kg sc, which was not considered excessive when compared with previous experiments. In addition, the median convulsive dose (CD_{50}) of lidocaine for mice was 289.4 ± 13.4 mg/kg sc[16], suggesting that 4 mg/kg sc was a safe dose for a mouse.

Several methods for assessment of apoptosis are available, including the detection of DNA damage, caspase assay, and mitochondrial analysis. Apoptosis should be determined by more than one method due to the possibility of misinterpretation using a single method. To evaluate brain apoptosis in this study, we used an IHC caspase-3 assay and TUNEL to determine DNA breakage. Caspase is a protease and caspase 3 is essential for the cleavage of protein producing the characteristics of apoptotic death. IHC detection of C-3A cells is considered an excellent means of mapping and quantifying neuroapoptosis [8, 10]. TUNEL is used to assay endonuclease cleavage products by end-labelling the DNA strand breaks enzymatically. This method is highly sensitive to detect the apoptotic nuclei and DNA fragmentation [17]. For quantitative evaluation of apoptosis, we chose sagittal section, as previous studies [8, 10, 11], that structures of each part of the brain could be well confirmed.

By IHC, not only entire brain but also particular part of brain such as the thalamus, cortex and caudate nucleus were examined. The density of C-3A positive cells in these 3 regions were greater than that in the entire brain, likely because these areas are most vulnerable to anaesthesia-induced cell

death [4]. The number of apoptotic cells were not statistically different between group C and group L in the cortex and the caudate nucleus, but there was difference in the thalamus. These results suggest that lidocaine may have potential neuro-apoptotic effect.

The other method for detection of apoptosis, the TUNEL assay, also suggested that not only isoflurane, but also lidocaine with midazolam, triggered apoptotic cell death, as the results of IHC study. In addition, the densities of TUNEL-positive fragmented nuclei in groups I were significantly greater than in group L. And group L exhibited more apoptotic cells than group C, although the difference was not significant (P= 0.05). These results were slightly different from IHC. TUNEL assay has been extensively used and known to be standard for the detection of apoptosis. Its great sensitivity sometimes results in false positive that does not matter in IHC study, one of the most specific tool for identifying apoptotic cells [18]. However, TUNEL assay is still considered to be standard and the findings of TUNEL staining corresponded with that of the cortex and thalamus by IHC in this study. Consequently, we cannot conclude that LA could avoid neuro-apoptosis during anaesthesia. Previous studies have also raised the possibility of LA-induced apoptosis [19-23].

Local anaesthetics are sodium channel blockers and are thought to be neurotoxic at certain concentrations. However, the mechanism of neurotoxicity is not related to the blockade of the sodium channel [19, 21].

Three mechanisms of LA-induced cell death have been suggested. First, lidocaine blocks Ca^{2+}-ATPase in the endoplasmic reticulum (ER), releasing Ca^{2+} from internal stores, and the change in Ca^{2+}homeostasis in the ER leads to neuronal apoptosis [24], as with isoflurane [25]. In one *in vitro* study, prevention of the lidocaine-induced increase in Ca^{2+} attenuated the lidocaine-induced neurotoxicity in the isolated rat dorsal root ganglion [23]. Second, activation of a kinase, such as p38 mitogen-activated protein kinase (MAPK), may mediate neurotoxicity [26]. In addition, one study reported that MAPK could trigger cellular apoptosis [27]. Third, mitochondrial injury and caspase activation [21] have been suggested to be mechanisms of the neuronal injury caused by local anaesthetics. The above-mentioned studies used cell culture models and isolated peripheral nerves or the spinal cord, not brain. However, 90% of LA infiltrated subcutaneously is known to be taken up by systemic circulation and reach high vascular organ such as brain [28]. Considering the toxicity of central nerve system related to LA has been important issue in regional anaesthesia especially in small children because of increased susceptibility [28], we cannot exclude the possibility of brain apoptosis caused by clinical dose of LA in vulnerable paediatric patients. It is clear that LA can induce neuronal apoptosis through various mechanisms [29]. Therefore, further evaluation is needed to determine the correlation of LA and brain apoptosis and the clinical significance of our findings.

There were some limitations to this study. First, cardiopulmonary function was not evaluated, such as by arterial blood gas analysis. Decreased

cardiopulmonary function may be related to brain hypoxia and ischaemia. We actually observed that some mice in group I had a slightly reduced respiration rate, which might be associated with significant hypercapnia and hypoxia, which needed an external stimulation. Second, to confirm the permanent neuronal loss by neural stem cells in developing period was not clear. Isoflurane anaesthesia is known to induce a loss of neural stem cell in dentate gyrus in immature animal that leads to permanent neurologic deficit [30]. Evaluation of apoptotic cells in dentate gyrus of hippocampus would be more specific to find the clinical correlation between LA and neurodegeneration. Lastly, we compared only the acute neuronal degeneration histologically, and not long term effect such as behaviour or learning disability.

In summary, LA may induce neuronal apoptosis in the immature brain, although not to the same degree as inhalational anaesthetics. Addition of midazolam to lidocaine has an effect on neuronal apoptosis similar to inhalational anaesthetics. More research is needed to investigate the degree and exact mechanism of brain apoptosis due to LA.

References

1. Beals, J.K., L.B. Carter, and V. Jevtovic-Todorovic, *Neurotoxicity of nitrous oxide and ketamine is more severe in aged than in young rat brain.* Ann N Y Acad Sci, 2003. **993**: p. 115; discussion 123-4.
2. Jevtovic-Todorovic, V., et al., *Prolonged exposure to inhalational anesthetic nitrous oxide kills neurons in adult rat brain.* Neuroscience, 2003. **122**(3): p. 609-16.
3. Jevtovic-Todorovic, V., et al., *Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits.* J Neurosci, 2003. **23**(3): p. 876-82.
4. Nikizad, H., et al., *Early exposure to general anesthesia causes significant neuronal deletion in the developing rat brain.* Ann N Y Acad Sci, 2007. **1122**: p. 69-82.
5. Slikker, W., Jr., et al., *Ketamine-induced neuronal cell death in the perinatal rhesus monkey.* Toxicol Sci, 2007. **98**(1): p. 145-58.
6. Kalkman, C.J., et al., *Behavior and development in children and age at the time of first anesthetic exposure.* Anesthesiology, 2009. **110**(4): p. 805-12.
7. Wilder, R.T., et al., *Early exposure to anesthesia and learning disabilities in a population-based birth cohort.* Anesthesiology, 2009. **110**(4): p. 796-804.
8. Young, C., et al., *Potential of ketamine and midazolam, individually or in combination, to induce apoptotic neurodegeneration in the infant mouse brain.* Br J Pharmacol, 2005. **146**(2): p. 189-97.
9. Zou, X., et al., *Potential neurotoxicity of ketamine in the developing rat brain.* Toxicol Sci, 2009. **108**(1): p. 149-58.
10. Young, C. and J.W. Olney, *Neuroapoptosis in the infant mouse brain triggered by a transient small increase in blood alcohol concentration.*

Neurobiol Dis, 2006. **22**(3): p. 548-54.

11. White, L.D. and S. Barone, Jr., *Qualitative and quantitative estimates of apoptosis from birth to senescence in the rat brain.* Cell Death Differ, 2001. **8**(4): p. 345-56.
12. Loepke, A.W., et al., *The effects of neonatal isoflurane exposure in mice on brain cell viability, adult behavior, learning, and memory.* Anesth Analg, 2009. **108**(1): p. 90-104.
13. Waite, A., et al., *Clinically relevant doses of lidocaine and bupivacaine do not impair cutaneous wound healing in mice.* Br J Anaesth, 2010. **104**(6): p. 768-73.
14. Lei, B., et al., *Effects of delayed administration of low-dose lidocaine on transient focal cerebral ischemia in rats.* Anesthesiology, 2002. **97**(6): p. 1534-40.
15. Miller, R.D., *Miller's anesthesia*, in *Miller's Anesthesia*, L.I. Eriksson, L.A. Fleisher, and J.P. Wiener-Kronish, Editors. 2010, Churchill Livingstone/Elsevier: Philadelphia, PA.
16. de Jong, R.H. and J.D. Bonin, *Local anesthetics: injection route alters relative toxicity of bupivacaine.* Anesth Analg, 1980. **59**(12): p. 925-8.
17. Blaylock, M., T. Engelhardt, and B. Bissonnette, *Fundamentals of neuronal apoptosis relevant to pediatric anesthesia.* Paediatr Anaesth, 2010. **20**(5): p. 383-95.
18. Duan, W.R., et al., *Comparison of immunohistochemistry for activated caspase-3 and cleaved cytokeratin 18 with the TUNEL method for quantification of apoptosis in histological sections of PC-3 subcutaneous xenografts.* J Pathol, 2003. **199**(2): p. 221-8.
19. Sakura, S., et al., *Local anesthetic neurotoxicity does not result from blockade of voltage-gated sodium channels.* Anesth Analg, 1995. **81**(2): p. 338-46.
20. Karon, B.S., et al., *Anesthetics alter the physical and functional properties of the Ca-ATPase in cardiac sarcoplasmic reticulum.* Biophys J, 1995. **68**(3): p. 936-45.

21. Johnson, M.E., et al., *Mitochondrial injury and caspase activation by the local anesthetic lidocaine.* Anesthesiology, 2004. **101**(5): p. 1184-94.
22. Johnson, M.E., et al., *Effect of local anesthetic on neuronal cytoplasmic calcium and plasma membrane lysis (necrosis) in a cell culture model.* Anesthesiology, 2002. **97**(6): p. 1466-76.
23. Gold, M.S., et al., *Lidocaine toxicity in primary afferent neurons from the rat.* J Pharmacol Exp Ther, 1998. **285**(2): p. 413-21.
24. He, H., et al., *Maintenance of calcium homeostasis in the endoplasmic reticulum by Bcl-2.* J Cell Biol, 1997. **138**(6): p. 1219-28.
25. Kindler, C.H., et al., *Volatile anesthetics increase intracellular calcium in cerebrocortical and hippocampal neurons.* Anesthesiology, 1999. **90**(4): p. 1137-45.
26. Lirk, P., et al., *In vitro, inhibition of mitogen-activated protein kinase pathways protects against bupivacaine- and ropivacaine-induced neurotoxicity.* Anesth Analg, 2008. **106**(5): p. 1456-64, table of contents.
27. Takeda, K. and H. Ichijo, *Neuronal p38 MAPK signalling: an emerging regulator of cell fate and function in the nervous system.* Genes Cells, 2002. **7**(11): p. 1099-111.
28. Dillane, D. and B.T. Finucane, *Local anesthetic systemic toxicity.* Can J Anaesth, 2010. **57**(4): p. 368-80.
29. Lirk, P., et al., *In vitro, lidocaine-induced axonal injury is prevented by peripheral inhibition of the p38 mitogen-activated protein kinase, but not by inhibiting caspase activity.* Anesth Analg, 2007. **105**(6): p. 1657-64, table of contents.
30. Zhu, C., et al., *Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents.* J Cereb Blood Flow Metab, 2010. **30**(5): p. 1017-30.

국문 초록

서론: 흡입마취제와 benzodiazepine 계열의 전신 마취 약제는 GABA 나 NMDA 수용체에 작용하면서, 시냅스 형성이 활발한 시기의 미성숙 뇌에 세포자연사 (apoptosis)를 일으킨다고 밝혀졌다. 국소마취제를 이용한 부위 마취는 전신마취제의 사용량을 줄일 뿐 아니라 전신마취의 대체 방법으로 사용될 수 있다. 하지만 국소마취제가 뇌의 세포자연사에 미치는 영향에 대한 연구는 아직 없다. 따라서 본 연구를 통해 뇌의 세포자연사에 국소마취제가 미치는 영향을 알아보고자 한다.

방법: 총 51마리의 생후 7일된 C57BL6 쥐를 대조군, lidocaine 군 (L군), lidocaine 및 midazolam 군 (LM군), isoflurane 군 (I군)으로 분류하였다. 대조군에는 생리식염수를 피하 주입하였다. L군에는 lidocaine 4 mg 을 피하 주입하였고, LM군은 추가로 midazolam 9 mg 을 피하 주입하였다. I 군은 6시간 동안 0.75% 의 isoflurane 에 노출시켰다. 실험 6시간 뒤, 모든 실험 쥐들을 경추 탈골 시키고 뇌를 적출하였다. 뇌의 세포자연사는 caspase-3 면역 염색과 TUNEL 염색으로 확인하였다.

결과: 전체적으로, LM군과 I군에서 caspase-3 활성화된 신경 세포의 밀도가 대조군에 비해 더 높게 측정되었다 (P = 0.039, P = 0.002). 시상에서는 대조군에 비해 L 군의 세포자연사가 더 많이 관찰되었다 (P = 0.001). 대뇌 피질에서 I 군의 세포자연사는 대조군 및 L군보다 더 많이 관찰되었다 (P < 0.001). 미상핵에서는 LM 군의 세포자연사가 대조군과 L군에 비해 유의하게 증가하였다 (P = 0.002, P = 0.006). TUNEL 염색으로 확인하였을 때, LM 군 및 I 군의 세포자연사가 대조군 보다 증가하였으며 (P = 0.006, P = 0.002), I 군의 경우는 L 군 보다 더 많은 세포자연사가 관찰되었다 (P = 0.036).

결론: 임상에서 Lidocaine의 사용이 미성숙 뇌에서 세포자연사를 일으키지 않는다고 결론 내리는 것은 성급하며, lidocaine 에 midazolam 을 추가한 경우 흡입마취제와 마찬가지로 뇌의 세포자연사가 증가한다. 국소마취제가 뇌의 세포자연사를 일으키는 정도 및 기전에 대해서는 더 많은 연구가 필요할 것으로 생각된다.

주요어 : 국소마취제, 뇌, 세포자연사

학 번 : 2011-21853